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RESEARCH PAPER

Neuroprotective molecular mechanisms of (−)-epigallocatechin-3-gallate: a reflective outcome of its antioxidant, iron chelating and neuritogenic properties

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Abstract Tea, the major source of dietary flavonoids, particularly the epicatechins, signifies the second most frequently consumed beverage worldwide, which varies its status from a simple ancient cultural drink to a nutrient component, endowed possible beneficial neuro-pharmacological actions. Accumulating evidence suggests that oxidative stress, resulting in reactive oxygen species generation, plays a pivotal role in neurodegenerative diseases, supporting the implementation of radical scavengers and metal chelating agents, such as natural tea polyphenols, for therapy. Vast epidemiology data indicate a correlation between occurrence of neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases, and green tea consumption. In particular, recent literature strengthens the perception that diverse molecular signaling pathways, participating in the neuroprotective activity of the major green tea polyphenol, (−)-epigallocatechin-3-gallate (EGCG), renders this natural compound as potential agent to reduce the risk of various neurodegenerative diseases. In the current review, we discuss the studies concerning the mechanisms of action implicated in EGCG-induced neuroprotection and discuss the vision to translate these findings into a lifestyle arena.

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Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid beta peptide
ALS	Amyotrophic lateral sclerosis
ARE	Antioxidant response elements
COMT	Catechol-O-methyltransferase
BBB	Blood–brain barrier
DA	Dopamine
EC	(–)-Epicatechin
ECG	Epicatechin-3-gallate
EGCG	(−)-Epigallocatechin-3-gallate
EGC	(–)-Epigallocatechin
MAPK	Extracellular mitogen-activated protein kinases
ERK1/2	Extracellular signal-regulated kinases
GAP-43	Growth associated protein-43
6-OHDA	6-Hydroxydopamine
HSP90	Heat shock protein 90
HIF-1	Hypoxia inducible factor-1
3-HK	3-Hydroxykynurenine
iNOS	Inducible nitric oxide synthase
MEK1	Mitogen-activated protein kinase 1
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	*N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
OS	Oxidation stress
PD	Parkinson's disease
PKC	Protein kinase C
PC12 cells	Rat pheochromocytoma cells
R-APO	R-apomorphine

ROS	Reactive oxygen species
GSH	Reduced glutathione
SAPPα	Soluble amyloid precursor protein
TNF-α	Tumor necrosis-alpha
TfR	Transferrin receptor
SNPC	Substantia nigra pars compacta
VEGF	Vascular endothelial growth factor
UPS	Ubiquitin proteasome system

Introduction

Epicatechin (flavan-3-ol compound) is commonly present in green tea and abundant with phenolic hydroxyl groups on its aromatic rings, which confer its antioxidant and iron chelating activities [130]. The importance of green tea catechins in enhancing cell resistance to oxidative stress (OS) goes beyond the simple scavenging and iron chelating activities and is mostly interesting in those pathologies, where OS and iron are involved [64, 104]. Numerous studies in the last decade have shown that green tea catechins have in vitro and in vivo activities in preventing and/or reducing the deleterious effects of oxygen-derived free radicals, associated with several chronic- and stress-related human diseases (see reviews [5, 74, 76, 100, 119, 135]). Several lines of evidence suggest that OS, resulting in reactive oxygen species (ROS) generation, either through an enzyme or metal catalyzed process, plays a pivotal role in clinical disorders, such as atherosclerosis, ischemia-reperfusion injury, cancer, stroke and neurodegenerative disorders [24, 33]. Oxidative damage to neuronal molecules and increased accumulation of iron in specific brain areas are considered major pathological aspects of Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) and thus, special interest has been assigned to the therapeutic feature of nutritional antioxidants and iron chelators in neurodegenerative diseases [23, 32, 74, 76, 97, 135].

A large number of epidemiological studies on PD and cognitive impairment described a moderate risk reduction in black (fermented), oolong (semi-fermented) and green (not-fermented) teas consumers, compared to non-tea drinkers [39, 84]. The favorable properties ascribed to tea consumption are believed to rely on its bioactive flavanol class-related catechins and their derivatives, demonstrated to act as radical scavengers and exert indirect antioxidant effects through activation of transcription factors, signaling regulators and antioxidant enzymes (for reviews see: [40, 76, 141]). In line with this, particular attention has been placed to study the neuroprotective action of antioxidants, iron-chelating and anti-inflammatory agents, green tea catechins and especially, the major component of green tea, (−)-epigallocatechin-3-gallate (EGCG) [75, 82, 117]. The revelation of novel molecular targets, possibly implicated in their neuroprotective action include: calcium homeostasis [45], extracellular mitogen-activated protein kinases (MAPK) [109] and protein kinase C (PKC) signaling pathways[58, 95], regulation of antioxidant enzymes [60], antioxidant response element (ARE) [8], cell death and cell survival genes and proteins, associated with mitochondrial function, such as Bcl-2 family members [49, 59, 137, 138], amyloid precursor protein (APP) processing pathway [58, 96] and iron regulators and sensors encoding genes and proteins, such as transferrin receptor (TfR) and hypoxia-inducible factor (HIF)-prolyl-hydroxylases [96, 134, 152].

This review aims to compile recent studies regarding the molecular mechanism of action, implicated in EGCG-induced neuroprotective and neuritogenic activities, which might be a reflective outcome of its effects on neuronal cell signaling pathways associated with antioxidant and iron chelating properties.

Green tea catechins: human neuronal efficacy and epidemiology studies

Green tea belongs to the *Theacease* family, derived from two plant varieties, *Camellia sinesis* and *Camellia assamica* [25], is the most widely consumed beverage aside from water in Japan, China and other Asian nations and presently becoming more popular in Western countries. The first scientific recognition of medicinal properties of green tea was in the sixteenth century, using extracts as therapeutic mean to cure fever, headache, stomach and articulation pain [124]. To date, green tea is generally regarded as safe by the U.S. Food and Drug Administration (FDA) [142] and has attracted attention for its health benefits, particularly with respect to its potential for preventing and treating cancer, cardiovascular diseases, inflammatory diseases, aging and neurodegenerative diseases [42, 56, 139].

Catechins account for 25–35% of the green tea dry extract and consist of eight polyphenolic flavonoid-type compounds, namely, (+)-catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechingallate (CG), (−)-epicatechin gallate (ECG), (+)-gallocatechin gallate (GCG) and EGCG. The most abundant catechin is EGCG, estimated that a cup of green tea (2.5 g of green tea leaves/200 ml of water) may contain 90 mg of EGCG and thus, thought to particularly contribute to the beneficial effects attributed to green tea, such as its neuroprotective properties [68, 142].

Several reports indicated that tea polyphenols can be attained in the brain and exert neuroprotective effect simply by drinking [60, 93, 94]. The metabolism of green tea catechins has been studied in various animal models and in

humans [62, 92]. Orally catechin administration to humans is absorbed, metabolized and excreted within 24 h [38]. Study with healthy green tea consumers revealed levels of EGCG, EGC and EC in the plasma in a dose-dependent concentration, varying between 0.2 and 2% of the ingested amount, with maximal concentration 1.4–2.4 h after ingestion [81]. In addition, after ingestion of 1.2 g of green tea solids (dissolved in two cups of warm water), the plasma samples collected at 1 h from human volunteers contain 46–268 ng/ml [62]. The half-life for EGCG is about 5 h and for EGC and EC, it varies between 2.4 and 3.4 h [145, 146]. EGCG is the only known polyphenol present in plasma in large proportion (77–90%) in a free form and its levels is reported to be higher in esophagus and large intestine, but lower in other organs, likely due to poor systemic absorption [5]. Preclinical studies in rat indicated that EGCG is mainly excreted through urine and bile [5]. Additionally, previous studies reported on instability of EGCG at the intestinal pH of 8.5 and a low bioavailability, while antioxidants (e.g. ascorbic acid and selenium) demonstrated to stabilize EGCG in the lumen and help to build up its concentration in the intestine. This suggests that poor absorption from the small intestine may play an important role in limiting EGCG bioavailability [21].

Previous study of oral green tea administration established the chemical identity of two major tea catechin metabolites, (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, in human urine and blood. These metabolites appeared to be formed by the intestinal flora in the human colon and then absorbed [62]. In addition, previously, it was reported that EC metabolites (epicatechin glucuronide and 3′-O-methylated epicatechin glucuronide) formed after oral ingestion of EC by rats, had gained entry to the brain [1]. Furthermore, study with labeled EGCG demonstrated a wide distribution of radioactivity in mouse organs including brain, after oral administration and small amount of [^{3}H] EGCG excretion in the urine after direct administration [123]. Recently, the absorption and pharmacokinetic of EGCG in various brain regions of adult and fetal rats have been demonstrated by oral and intravenous administration, indicating that EGCG is the most abundant catechin in brain tissue [65, 68] and may potentially penetrate through the blood–brain barrier (BBB) [12]. In vitro model of BBB demonstrated that various flavonoids and some metabolites were able to traverse the BBB and that the potential for permeation was consistent with compound lipophilicity [149].

Albeit the uncertainty on the capacity of green tea catechins to penetrate the brain, green tea was suggested to inversely correlate with the incidence of brain aging, dementia and neurodegeneration, such PD and AD. Previous epidemiological studies have shown a reduced risk of PD associated with consumption of 2 cups/day or more of black tea [7]. In support, Tan et al. [126] found an inverse association between black tea and PD, based on a 12-year prospective study of over 63,000 men and women, which was due to black tea ingredients separate from its caffeine content. In a cross-sectional study, aimed at investigating an association between consumption of green tea and cognitive function in elderly Japanese subjects, it was found that higher consumption of green tea is associated with lower prevalence of cognitive impairment [55]. Despite the fact that the prevalence of PD is much lower in tea consumers, the association of green tea drinking and risk of AD and other neurodegenerative diseases is not well established. No case–control study has been accomplished that points to a beneficial effect associated with tea consumption in AD, although recently Ng et al. [84] performed analysis of green tea intake by comparing baseline data from 2,501 participants and 1,438 cognitively intact participants from 2-year follow-up of Chinese cohort in the Singapore Longitudinal Ageing Study. Green tea was inversely associated with cognitive impairment, but not with cognitive decline, possibly due to the small number of green tea drinkers in this cohort. These findings emphasize the importance of well-designed controlled studies to assess a risk reduction of PD and AD in consumers of green tea. Indeed, a randomized, double-blind and efficacy study in Beijing China is under completion (2009), to determine the safety, tolerability and potential neuroprotective effects of a green tea polyphenol enriched preparation, in de novo PD patients without taking any anti-Parkinsonism drug (sponsored by the Michael J. Fox Foundation for Parkinson's Research). In accordance, another clinical efficacy study (sponsored by Charite University, Berlin, Germany) is aimed to evaluate whether a green tea extract containing 95% EGCG, given daily as oral medication over a period of 12 months, has anti-inflammatory and neuroprotective properties in patients with relapsing–remitting multiple sclerosis assessed by magnetic resonance imaging and clinical examination (estimated study completion date is April 2009; U.S. FDA resources).

EGCG neuroprotective mechanism of action

Preclinical studies

Although evidence in human studies is limited, there are accumulated studies and reports on in vitro and animal models of aging and aged-related neurodegenerative diseases, demonstrated protective effects of green tea catechins. Neuroprotective in vivo studies employing the Parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown that both

green tea extract and EGCG possess highly potent activities in preventing mice striatal dopamine (DA) depletion and substantia nigra (SN) dopaminergic neuron loss [60]. One possible mechanism underlying the effectiveness of green tea and EGCG against MPTP neurotoxicity may involve its catechol-like structure, as it is known that catechol-containing compounds are potent radical anti-oxidants and ferric ion chelator, such as R-apomorphine (R-APO) [27, 28, 30]. The catechol structural resemblance may account for a recently reported inhibitory effect of green tea polyphenols on [^{3}H] DA uptake by presynaptic transporters. This inhibition was suggested to block the metabolic product of MPTP, the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) uptake (because of competition for the vesicular transporter) thereby protecting DA-containing neurons against MPP^+-induced injury [89]. In vitro studies also demonstrated inhibition of MPP^+ and 6-hydroxydopamine (6-OHDA)-induced neurotoxicity by EGCG [59]. Furthermore, EGCG, at a low IC_{50} concentration (0.2 μM), inhibited the activity of the enzyme catechol-O-methyltransferase (COMT) in rat liver cytosol homogenates [69]. DA and related catecholamines are physiological substrates of COMT. The COMT inhibitors, entacapone and tolcapone, clinically prescribed to PD-affected individuals, dose-dependently inhibited the formation of the major metabolite of levodopa, 3-O-methyldopa, thereby improving its bioavailability in the brain [15]. In addition, iron accumulation has been implicated in a range of neurodegenerative disorders [111] and iron has been reported to accumulate in the neurons and microglia in SN of PD patients [98, 151]. Thus, the implication of the iron chelation property of EGCG in neuroprotection has been strengthened by the observations that both MPTP and 6-OHDA significantly increased iron levels in SN pars compacta (SNPC) of mice, rats and monkeys [79, 127].

Although AD epidemiological studies did not report any established outcome relative to green tea consumptions, in vitro observations showed that EGCG prevented amyloid beta peptides (Aβ)-induced neurotoxicity [11, 58], and EC reduced nascent Aβ fibrils, elongation of the fibrils and destabilization of the formed assemblies [85]. In addition, EGCG was able to regulate the proteolytic processing of APP under in vivo and in vitro conditions [58, 96], suggesting that green tea polyphenols might be potentially promising therapeutic agents not only for PD, but also for AD. EGCG promoted the non-amyloidogenic α-secretase pathway of APP in neuronal cell cultures resulting in a consequential augment in soluble APPα (sAPPα) [58]. In addition, long-term treatment of mice with EGCG resulted in decreases in cell-associated, full-length APP levels, as well as increases in the sAPPα levels in the hippocampus [58]. New supportive data came from a study conducted in an Alzheimer's transgenic mice model ("Swedish" mutant APP overexpressing, APPswTg), showing that EGCG promoted the generation of sAPPα and decreased Aβ levels and plaques via promotion of the non-amyloidogenic α-secretase proteolytic pathway [93, 94]. Recently, long-term administration of EGCG provided prophylactic benefits on rat spatial cognitive learning impairment caused by Aβ cerebral ventricle infusion [37], as well as prevented lipopolysaccharide-mediated neuronal cell death and memory impairment of mice, possibly through reduction of Aβ levels via inhibition of β- and γ-secretases [57].

Supplementary preclinical models of other neurodegenerative diseases demonstrated that EGCG induced the prolongation of the symptom onset and life span and attenuated death signals in ALS mice model, expressing the human G93A mutated Cu/Zn-superoxide dismutase (SOD1) gene [54, 144]. Additionally, EGCG has been shown to be neuroprotective in aged rats [122] and in animal models of focal and global brain ischemia [91, 124, 125].

ROS regulation and iron chelating activities

It is well established that catechins possess free radical scavenging properties and act as biological antioxidants [82, 83, 90]. Catechins can scavenge both superoxide and hydroxyl radicals, as well as the 1,1-diphenyl-3-picrylhydrazyl radical, peroxyl radicals, nitric oxide, carbon-center free radicals, singlet oxygen and lipid free radicals, and peroxynitrite by preventing the nitration of tyrosine [30, 68, 80, 82, 90, 104, 121, 153]. Additionally, these compounds can chelate metal ions, such as copper (II) and iron (III) to form inactive complexes and prevent the generation of potentially damaging free radicals [110]. Catechins have been found to be more efficient radical scavengers than vitamin E and C [82, 90].

In the majority of the reports, EGCG was shown to be more efficient as a radical scavenger than its counterparts ECG, EC and EGC, which may be attributed to the presence of the trihydroxyl group on the B ring and the gallate moiety at the 3′ position in the C ring (Fig. 1) [31, 82, 83]. Previously, EGCG was shown to attenuate hydroxykynurenine (3-HK)-induced cell death and the increase in ROS concentrations and caspase-3 activity in neuronal culture, presumably via its antioxidant activity [48]. In rat brain tissue, green and black tea extracts were shown to inhibit lipid peroxidation promoted by iron-ascorbate in brain mitochondrial membranes [61]. A similar effect was also reported using brain synaptosomes, in which the four major green tea catechins were shown to inhibit iron-induced lipid peroxidation [30]. In this regard, it has been shown that EGCG attenuated paraquat-induced microsomal lipid peroxidation and increased the survival rate of paraquat-poisoned mice [41, 148]. In addition, Higuchi et al. [41]

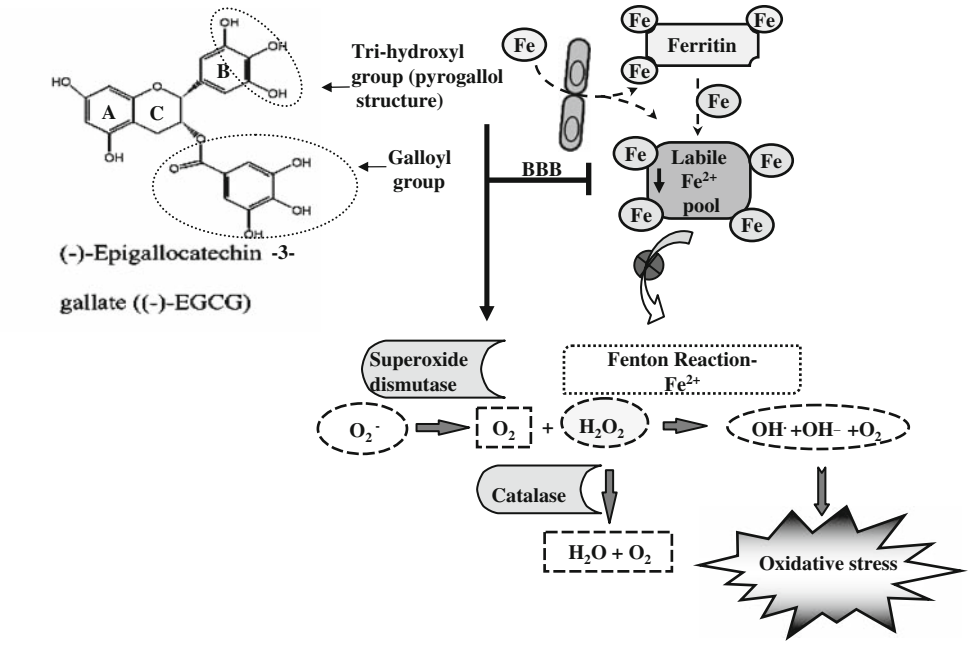

Fig. 1 Schematic diagram illustrating the antioxidative and iron chelating activities of EGCG. The neuroprotective effects of EGCG may involve inhibition of Fenton reaction and up-regulation of antioxidant enzymes, such as superoxide dismutase and catalase, resulted in attenuation of oxidative stress

suggested that EGCG inhibited paraquate-induced malondialdehyde production in rat liver microsome system containing $FeSO_4$ by two possible mechanisms: one was by scavenging of superoxide radicals, which were responsible for the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) catalyzed by the Fenton reaction. The other, was through iron-chelating activity, given that the inhibition disappeared when excessive amount of $FeSO_4$ was added to the reaction, indicating that EGCG inhibited iron driven lipid peroxidation by pulling out available iron. Thus, the protective effect of EGCG against neurological diseases may involve its radical scavenging and iron chelating activities. Additionally, the inhibition of enzymes, whose activity may promote OS, or an increase of antioxidant enzyme activities, might have beneficial significance to EGCG neuroprotection. Indeed, previous reports demonstrated that EGCG was found to elevate the activity of two major antioxidant enzymes, superoxide dismutase (SOD) and catalase in mice striatum [60] (Fig. 1).

The ability of green tea catechins and in particular EGCG, to chelate metal ions, such as iron and copper, may contribute to their antioxidant/neuroprotective activity by inhibiting transition metal-catalyzed free radical formation. The two points of attachment of transition metal ions to flavonoid molecules are: the o-diphenolic groups in the 3′,4′-dihydroxy positions in the B ring, and the keto structure 4-keto, 3-hydroxy or 4-keto and 5-hydroxy in the C ring of the flavonols (Fig. 1) [129, 130]. The ability of green tea catechins to act as relatively potent metal chelators [26, 30] may be of major significance for the treatment of neurodegenerative diseases, since iron accumulation has been shown in neurodegenerative brain areas [97]. Most importantly, green tea was reported not to affect iron absorption in healthy human subjects [10]. The localization of iron and ferritin in PD patients is restricted to specific brain areas [47, 97, 118] in the SNPC, but not the reticulata [47]. Similarly, AD pathogenesis is associated with iron accumulation and is linked to the characteristic neocortical Aβ deposition, phosphorylation of tau and tangle formation, which may be mediated by abnormal interaction with excess free-chelatable iron [22, 132]. Ionic iron can, in turn, participate in the Fenton reaction with subsequent generation of ROS, initiating the processes of OS and inflammatory cascade, resulting in the production of cytotoxic cytokines (tumor necrosis-alpha (TNF-α), interluekins-1 and -6) in the microglia and surrounding neurons [78, 103] and activation of transcription factors and nuclear factor-kappa B (NF-κB) [66, 107]. Indeed, a marked increase in NF-κB immunoreactivity was found in the nucleus of dopaminergic neurons of the Parkinsonian SNPC, compared to normal brains [43]. EGCG was found to inhibit the nuclear translocation of NF-κB in in vitro systems: immunofluorescence and electromobility shift assays showed that introduction of green tea extract before 6-OHDA inhibited both NF-κB nuclear translocation and binding activity in human neuroblastoma SH-SY5Y cells [61, 71]. Furthermore, these reduced activity of NF-κB by EGCG and the theaflavin-3,3′-digallate polyphenol from black tea was associated with inhibition of lipopolysaccharide (LPS)-induced TNF-α production [147] and the enzyme inducible nitric oxide synthase (iNOS) [66, 67, 88], which is responsible for the production of the short-live free radical, nitric oxide, in activated macrophages.

Interestingly, recent studies have identified a novel link between iron and AD, associated with an enhancement of endogenous APP translation and subsequent Aβ formation, via activation of an iron responsive element (IRE-type II) in the 5′ untranslated region (UTR) of APP mRNA [99]. Notably, recent study have demonstrated a significant increase of reactive astrocytosis and iNOS immunoreactivity, which was accompanied by neuronal damage in the temporal cortex and hippocampus of rats injected with Aβ (25–35) [63]. These findings opened a new potential therapeutic avenue aimed at reducing amyloidosis with radical scavenger and iron-chelating drugs that modulate APP mRNA translation. In support, a recent in vitro study demonstrated that EGCG reduced full-length APP in SH-SY5Y cells without altering APP mRNA levels, accompanied by dose-dependent increase in the level of the iron metabolism-related protein, TfR [96], which share also the consensus sequence for an IRE in the 3′-UTR of its mRNA [17]. Exogenous iron supplementation reversed EGCG effects, suggesting a post-transcriptional action, presumably by the mechanism of chelating intracellular iron pools (Fig. 1). This is further supported by the observation that EGCG suppressed translation of a luciferase reporter gene driven by the IRE-type II-containing sequences of APP [96]. Furthermore, it was found that EGCG markedly reduced secreted Aβ levels in the conditioned medium of Chinese hamster ovarian cells, overexpressing the "Swedish" mutated APP (CHO/ΔNL) [96] and in primary neuronal cells derived from transgenic mice bearing the APP "Swedish" mutation [94].

More recently, Friedlich et al. [19] have described a putative IRE in the 5′-UTR of PD-related α-synuclein mRNA and predicted that this RNA structure may have a potential to function as a post-transcriptional regulator of its protein synthesis in response to iron and redox events, resembling the pattern seen with APP and the iron-associated proteins, ferritin and TfR. This finding can explain, in part, previous observation demonstrating that EGCG prevented iron-dependent up-regulation of α-synuclein in the SNPC of MPTP-treated mice, resulting in neuroprotection of SN dopaminergic neurons [72]. Thus, the radical scavenging and free-iron chelating activities of green tea catechins may directly influence aggregation and deposition of either Aβ or α-synuclein in brains of AD and PD patients, respectively.

Regulation of hypoxia inducible factor (HIF)-1 alpha pathway

An emerging target for neuroprotection associated with iron chelation implicates the activation of a hypoxia signal transduction pathway that culminates in the stabilization of the transcriptional activator HIF-1 and increased transcription of genes mediating compensatory survival processes in response to OS [46, 113]. The presence of HIF-1 within the cells is under the strict control of a class of iron-dependent and oxygen-sensor enzymes, named the HIF prolyl-4-hydroxylases [46]. This family of enzymes hydroxylates critical proline and asparagine residues in HIF upon high oxygen levels and iron overload, targeting it for degradation by the ubiquitin proteasome system (UPS). In this scheme, iron chelators would stabilize HIF-1 alpha, which in turn would heterodimerize with its partner, HIF-1 beta in the nucleus, bind to an hypoxia responsive element in regulatory genes and transactivate the expression of established protective genes, including the angiogenic vascular endothelial growth factor (VEGF), erythropoietin, $p21^{waf1/cip1}$, glucose transporter-1 and the glycolytic enzymes aldolase and enolase-1 [114, 150]. Indeed, EGCG and ECG were shown to induce HIF-1 alpha protein and HIF-1 activity and increase mRNA expression levels of glucose transporter 1 (GLUT-1), VEGF, and $p21^{waf1/cip1}$, whereas this effect was blocked by iron and ascorbate, indicating that these catechins may activate HIF-1 through the chelation of iron [128, 154]. Applying a neurorescue paradigm in neuronal culture, we have recently found that EGCG decreased mRNA transcript and protein levels of the beta-subunit of prolyl-4-hydroxylase and the protein levels of two molecular chaperones, which are associated with HIF-regulation/degradation, the immunoglobulin-heavy-chain binding protein, BiP and the heat shock protein 90 (HSP90) [133, 134] (Fig. 2). In support, previous finding demonstrated that EGCG directly binds and inhibits HSP90 in mouse hepatoma cells [87]. Inhibition of HSP90 is considered a requirement for the rapid hypoxic stabilization of HIF-1 alpha, which otherwise might be degraded by unspecific pathway [44]. Thus, it is possible that the protective effect of EGCG under OS/hypoxic conditions may combine the suppression of hydroxyl radical formation via Fenton chemistry, as well as inhibition of iron-dependent prolyl hydroxylase.

Another link between hypoxia and iron is reflected by the hypoxic-mediated positive regulation of the iron regulatory proteins, IRP1 and IRP2 and consequential transactivation of their target mRNAs, ferritin and TfR. Interestingly, the free iron-induced proteasomal-mediated degradation of IRP2 involves also activation of a prolyl hydroxylase and is inhibited by iron chelators [35, 36, 131]. Thus, it is possible that IRP2 is a substrate for this enzyme, in a similar way as HIF, signaling it for protein degradation.

The reduction in the chelatable iron pool by EGCG may result in the inhibition of prolyl hydroxylase activity and consequently, in the concerted activation of both HIF and IRP2. As HIF-1 and IRPs coordinate the expression of a wide array of genes, involved in cellular iron homeostasis,

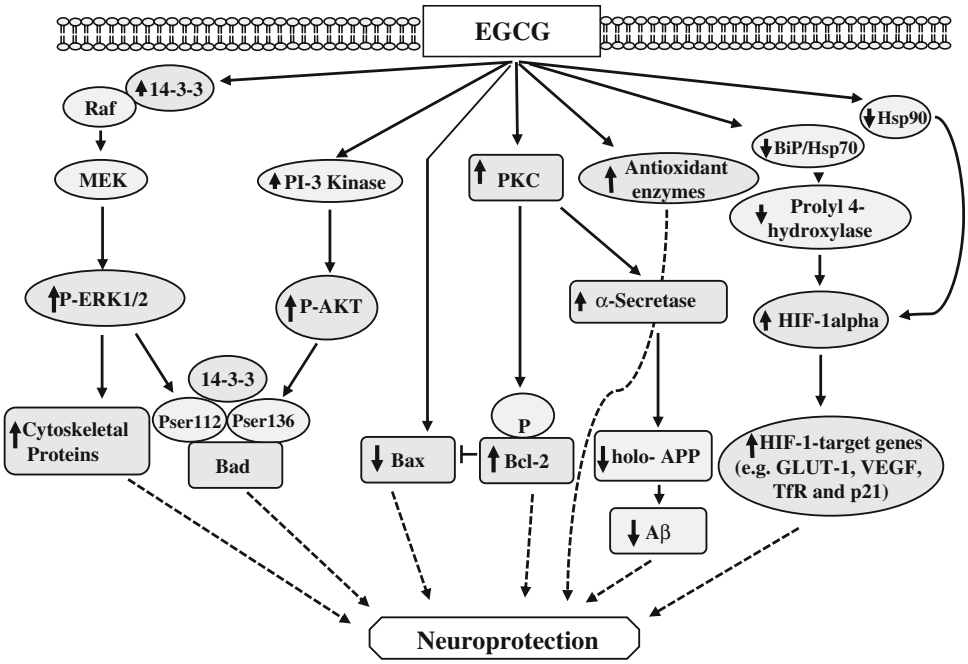

Fig. 2 A proposed schematic model of the neuroprotective mechanism of action of (−)-epigallocatechin-3-gallate (EGCG). The diagram demonstrates the potential molecular pathways involved in the multifunctional effects of EGCG in neuronal tissues. Full discussion is in the text

survival and proliferation [112], their activation could be of major importance in novel therapeutic strategy of neurodegenerative diseases. In support, recent findings suggest the application of low molecular weight or peptide inhibitors of the HIF prolyl 4-hydroxylases as novel neurological therapy [113].

Regulation of cell signaling pathways promoting neuronal outgrowth

Emerging evidence suggests that the iron chelating and antioxidant activities of green tea catechins cannot be the exclusive mechanism responsible for their neuroprotective action, but rather, their ability to alter signaling pathways may significantly contribute to the cell survival effect [120, 140]. Modulation of cellular survival and signal transduction pathways has significant biological consequences that are important in understanding the various pharmacological and toxicological responses of antioxidant drugs. A number of intracellular signaling pathways have been described to play central functions in EGCG-promoted neuronal protection against a variety of extracellular insults, such as the MAPK [115, 134, 143], PKC [14, 59, 95, 133] and phosphatidylinositol-3-kinase (PI-3 kinase)-Akt pathways [50, 53, 73]), as described in Fig. 2. Given the critical role of MAPK pathways in regulating cellular processes that are affected in neurodegenerative diseases, the importance of MAPKs as transducers of extracellular stimuli into a series of intracellular phosphorylation is being increasingly recognized. OS seems to be a major stimulus for MAPK cascade, which might lead to cell survival/cell death. Previous in vitro studies demonstrated the potency of EGCG to induce ARE-mediated defensive genes and MAPKs pathways, including various cell survival signaling regulators, p44/42 ERK 1/2, JNK and p38 MAPK [8, 86]. The role of ERK1/2 signaling seems to be connected to attenuation of neuronal death and cellular injury by OS [106]. EGCG counteracted the decline in ERK1/2 induced by 6-OHDA [59] and induced phosphorylation of ERK1/2 in serum-deprived SH-SY5Y cells [134].

(−)-EGCG activity also involves the intracellular signaling mediator, PKC [58, 95], thought to have an essential role in the regulation of cell survival and programmed cell death [16, 70]. A rapid loss of neuronal PKC activity is a common consequence of brain neurodegeneration [4, 6]. The induction of PKC activity in neurons by EGCG (1–10 μM) is thought to be a prerequisite for neuroprotection against several neurotoxins, such as Aβ [58], serum withdrawal [73, 95, 133] and 6-OHDA [59] since inhibition of PKC phosphorylation completely abolished the protection induced by EGCG and by the PKC activator, phorbol 12-myristate 13-acetate (PMA). These in vitro results were supported by a previous report [58], demonstrating that EGCG caused a significant increase of the levels of PKC isozymes, α and ε, in the membrane and cytosolic fractions of mice hippocampus. These isoforms play a crucial role in cell survival and differentiation pathways [29] and may be involved in APP regulatory processing associated with the pathogenesis of AD [2, 116]. Indeed, previous studies in brains of AD patients demonstrated reduction of PKCε activity [77].

The mechanism by which PKC activation leads to neuroprotection begins to be elucidated. Studies with extra-neuronal tissues support a role for PKCα as a kinase of the antiapoptotic Bcl-2, probably through direct or indirect phosphorylation of this cell survival protein [101]. Neuroprotective experimental studies demonstrated that the protective effect of EGCG was associated with a reduced levels of the apoptotic markers, cleaved caspase 3; its downstream cleaved substrate poly-ADP-ribose-polymerase (PARP) and Bad [95, 137, 138]. This is supported by the observation that EGCG could not overcome neuronal death under PKC pathway blockade, suggesting that this cascade is essential for the neuroprotective and neurorescue effects of EGCG [95].

Recently, we have identified a novel pathway in the neuroprotective mechanism of action of EGCG, which involves a rapid PKC-mediated degradation of Bad protein by the ubiquitin UPS in SH-SY5Y cells [49]. Bad has been suggested to link survival signals to the mitochondrial cell death machinery. Thus, the newly described role of Bad during the initial response to EGCG-induced cell signaling may illuminate the mechanism of neuroprotective/neurorescue action of EGCG. In addition, EGCG was shown to induce a rapid translocation of the isoform PKCα to the membrane compartment in human astroglioma or rat pheochromocytoma PC12 cells [52, 95], as well as upregulation of PKCε mRNA expression and a concentration-dependent activation of PKCε in serum-deprived in SH-SY5Y cells [133]. These findings are supported by animal studies showing that 2 weeks oral consumption of EGCG prevented the extensive depletion of PKCα and counteracted the robust increase of Bax protein in the striatum and SNPC of mice intoxicated with MPTP [73].

A previous study in human epidermal keratinocytes indicated that EGCG promoted cell survival by increasing the ratio of the pro-survival Bcl-2 to pro-apoptotic Bax and inducing phosphorylation of Bad through ERK and AKT signaling pathways [13]. Using mitogen-activated protein kinase 1 (MEK1) inhibitor (PD98059), EGCG induced only the phosphorylation of serine (Ser)136 of Bad, while using PI-3 kinase inhibitor (LY294002), EGCG induced only the phosphorylation of Ser112 of Bad. These results indicated that EGCG affects both the ERK pathway, which is involved in phosphorylation of Bad at Ser112 and the PI-3 kinase/AKT pathway, involved in phosphorylation of Bad at Ser136 (Fig. 2). Nonetheless, a study with high concentrations with EGCG reported cell proliferation arrest of tumor cells and inhibition of ERK1/2 and AKT phosphorylation, which was associated with reduced phosphorylation of Bad [102]. This biphasic mode of biological activity of EGCG relies on its concentration-dependent window of pharmacological action: EGCG exhibits pro-oxidant and pro-apoptotic activity at high concentrations, which are responsible for its anti-cancer-cell death effect, while lower doses exert neuroprotection against a wide spectrum of neurotoxic compounds [137, 138]. A biphasic mode of action has been described for most of the typical radical scavengers and antioxidants, such as ascorbic acid (vitamin C) [34] and iron chelators, such as R-APO [20]. When SH-SY5Y cells were challenged with 6-OHDA or reduced content of serum, a low concentration of EGCG (<10 μM) abolished the induction of proapoptotic-related mRNAs and the decrease in Bcl-2, Bcl-w and Bcl-xL [61, 137, 138]. The neuroprotective effect of EGCG is thought to be mediated through down-regulation of pro-apoptotic genes, as shown for mdm2, caspase-1, cyclin-dependent kinase inhibitor p21 and TNF-related apoptosis-inducing ligand, TRAIL, rather than up-regulation of anti-apoptotic genes [137, 138]. In support, a recent study has shown that at nanomolar concentrations, EC stimulated MAPK and PI-3 kinase signaling pathway, cAMP-response element binding (CREB) protein phosphorylation and ERK-dependent cAMP responsive element activity in primary cortical neurons, while at micromolar concentrations, EC-mediated activation of protein kinase pathways was lost and there was an inhibition of CREB phosphorylation [108].

In addition, a recent proteomic study [134] has demonstrated that EGCG increased the levels of the cell signaling binding protein, 14-3-3 gamma. By their interaction with more than 100 binding partners, 14-3-3 protein family members modulate the action of proteins that are involved in cell cycle and transcriptional control, signal transduction, intracellular trafficking, regulation of ion channels and expression of cytoskeletal components [3]. In this regards, the neurorescue/neuroprotective activity of EGCG may be associated with the induction of 14-3-3 gamma, interacting with kinases of the PKC pathway and Bad and consequently preventing neuronal death (Fig. 2) [49, 74, 95, 134]. A previous study has demonstrated that overexpression of 14-3-3 gamma contributed to the regulation of the dynamics of glial fibrillary acidic protein (GFAP) filaments, which may facilitate the stability of the cytoskeleton and thus, play a specific neuroprotective role in the brain of AD patients [18]. In fact, recent proteomic analysis showed that EGCG dose-dependently increased the expression levels of various stabilizer proteins of chromatin organization and DNA, histone H1 member 4 and cytoskeletal proteins, such as the actin binding protein, tropomyosin 3 and beta-tubulin IV [134]. Since cytoskeletal proteins play a crucial role in promoting neurite outgrowth [105], these results suggest that the induction of structural proteins by EGCG is associated with its differentiation features, including neurite extension, cell body elongation and up-regulation of the growth associated protein-43 (GAP-43) [95, 133]. These findings support the

assumption that in addition to antioxidant and iron chelating activities, complementary mechanisms are involved in the neuroprotective effect of EGCG.

Conclusions and viewpoints

Two main aspects are significantly contributing to the raising concept viewing green tea consumption of relevance to brain health: the factors and events that influence the incidence and progression of PD and AD are becoming better defined and understood; in parallel, the experimental evidence documenting the neuroprotective properties of green tea catechins, both in cell culture and animal studies are persistently increasing. It becomes evident that syndromes, such as AD and PD will require multiple drug therapy to address the varied pathological aspects of the disease [136]. Therefore, the poly-pharmacological activities of green tea catechins may be of significance for neuroprotection. Earlier, viewed as a mere anti-inflammatory and antioxidant, EGCG is at the present time considered a multimodal acting molecule, invoking various cellular neuroprotection/neurorescue mechanisms including iron-chelation, scavenging of oxygen and nitrogen radical species and activation of PKC signaling pathway and pro-survival genes (Figs. 1, 2). Its non-toxic, lipophilic nature, and thus presumably brain permeable, is advocated for "remove iron" from those brain areas, where it preferentially accumulates in neurodegenerative diseases. Additionally, the chelation of reactive free-iron pool by EGCG and consequent reduction in APP translation would contribute to decrease Aβ generation/fibrillization, which together with the promotion of the non-amyloidogenic pathway and induction of neurite outgrowth, may converge in a slowdown in the process of neuronal loss in AD.

Another novel therapeutic approach may involve drug combinations, mixing target-acting compounds, thus providing a practical way to design specific polypharmacology. The complex symptomatology of neurodegenerative diseases often necessitates the use of more than one multifunctional drug. Over the years, it has become evident that some combinations do induce a favorable clinical response, not achieved by each of the drugs given alone [51]. Currently, choice of drug combinations is based on a trial and error paradigm guided by the clinical response. Understanding the biological principles, by which the combined treatments act, would provide insights into the pathological mechanisms of neurodegenerative disorders and enable a more "rational" selection of drugs. Indeed, recent narrative regimen study described a combined treatment of memantine, the first drug in a novel class of AD medications, and tea polyphenol, in excitotoxic mouse brain injury demonstrating significant neuroprotective effects of the combined treatment, compared with memantine and tea polyphenol treatment alone, including reduction in increased synaptosomal ROS and calcium concentration and attenuation of decreased anion channel ATPase activity and mitochondrial potential, accompanied with improved locomotor activity [9].

Our vision is to translate preclinical and clinical findings of green tea catechins into a lifestyle arena. Thus, future efforts in the understanding of the neuroprotective/neurorescue mechanism of action of EGCG must concentrate on deciphering specific cellular targets, and signaling cascades. Further preclinical studies are needed to clarify if EGCG and its metabolites, at sufficient concentrations, can reach the brain and regulate cell-signaling pathways and whether these effects can be successfully translated into prospect human studies to affect the progression of neurodegenerative disorders.

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